Remarks

Claim 94 has been added. The application presently contains claims 1-10, 13, 20, 22, and 91-94. No new matter is added by this amendment. Support for the amendment may be found in the original claims and throughout the specification, *e.g.*, at page 22 lines 24 through 32. Applicants respectfully request entry of the foregoing amendment and submit that this amendment puts the application in condition for immediate allowance or appeal.

1. Rejections under 35 U.S.C. § 102

Claims 1-7, 9, 10, 13, 20, and 91-93 were rejected under 35 U.S.C. §102 (e) as being anticipated by Lee *et al.* (U.S. Patent Number 6,020,169) (hereafter "Lee"). Final Office Action mailed January 13, 2003 ("Final Action") at page 2. However, in order to support an anticipation rejection under 35 U.S.C. §102, the Office must demonstrate that each and every element of a claimed invention is disclosed within a single prior art reference. *In re Bond*, 15 U.S.P.Q.2d 1566, 1567 (Fed. Cir. 1990). Indeed, the reference must describe an applicant's claimed invention sufficiently to have placed a person of ordinary skill in the art in the field of the invention in possession of it. *See In re Paulson,* 31 U.S.P.Q.2d 1671 (Fed. Cir. 1994). The Office has not demonstrated that Lee teaches each and every element of the claimed invention, and therefore the anticipation rejection is improper.

Claims 1-7, 9-10, 13 and 20

The Office alleges that "Lee shows that 1% of Total protein is the newly expressed cytokine." Final Action at page 6. Therefore, the Office argues that Lee anticipates claims 1-7, 9-10, 13 and 20, which recite limitations that the cytokine accumulates to a level greater than 1% of the total soluble protein. Applicants respectfully traverse this rejection.

As previously stated in Applicants' First Response (dated December 6, 2002), Lee is deficient as an anticipatory reference because whatever else it may teach or suggest,

Lee fails to teach or suggest the accumulation of an expressed cytokine to a level greater than 1% of the total soluble protein. However, the Office contends that clone 81 of the Lee reference (shown in Figure 11) "produced over 1000 ng IL-4 per gram of Calli, which inherently represents more than 1% of the total soluble protein." Final Action at page 3.

Applicants respectfully submit that no basis has been provided for the Office's suggestion that 1000 nanograms of IL-4 per gram of Calli inherently represents more than 1% of the total soluble protein. To establish inherency "the Office must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990). To be found inherent in an anticipating reference, an unstated element must exist as a matter of scientific fact and flow naturally from the elements expressly disclosed in the prior art reference. *Hughes Aircraft Co. v. U.S.*, 8 USPQ2d 1580, 1583 (Ct. Cl. 1988). Compare *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999); *Atlas Powder Co. v. Ireco Inc.*, 190 F.3d 1342, 1347 (Fed. Cir. 1999); *Abbott Laboratories v. Geneva Pharmaceuticals, Inc.*, 182 F.3d 1315 (Fed. Cir. 1999). The Office has not provided any such technical reasoning or scientific fact, and thus has not met its burden of proof.

The Office alleges that Lee shows "1% of total protein is the newly expressed cytokine" but fails to explain how it has arrived at this value. Final Action at page 6. To properly determine the percentage of total protein attributable to the cytokine, the ratio of grams total protein per gram calli must be known. Lee does not disclose or teach this ratio, nor does it disclose or teach a conversion factor to calculate this ratio. Rather, the Office provides a line of reasoning which equates the amount of cytokine per gram of Calli to amount of cytokine per total soluble protein. This equation is tantamount to comparing apples to oranges.

Applicants respectfully submit that, as a matter of scientific fact, 1000 nanograms IL-4 per gram of Calli is far from 1% of the total soluble protein. The percentage of cell

weight that is protein depends on cell type, culture methods, and protein measurement methods. As an approximate value, the well-known textbook Molecular Biology of the Cell suggests that total protein is around 10 to 20% of total cell weight. Alberts *et al.*, Molecular Biology of the Cell 88 (2d. ed. 1989) (attached hereto as Exhibit A). Using a generous assumption of cell composition being 20% protein, Applicants calculate the percentage of IL-4 produced by clone 81 in Lee as follows:

$$\frac{1 \text{ g calli cells}}{0.2 \text{ g total protein}} \times \frac{1000 \text{ ng IL-4}}{1 \text{ g calli}} \times \frac{1 \times 10^{-9} \text{ g}}{1 \text{ ng}} = \frac{0.000001 \text{ g IL-4}}{0.2 \text{ g total protein}}$$

$$\frac{0.000001 \text{ g IL-4}}{0.2 \text{ g total protein}} \times 100 = 0.0005 \text{ \% of total protein is IL-4}$$

Applicants also present an alternative calculation, based on a conversion factor from Gao and Lee, Biotechnol. Progress 8(4):285-90 (1992) (hereafter "Gao") (attached hereto as Exhibit B). Gao presents data indicating that there is about 0.00599 grams of protein per 1 gram fresh cell weight of tobacco NT-1 cells (the same cells used by Lee).[1] Whether or not the protein data in Gao is soluble protein data or total protein data, Gao refutes the Office's reasoning. Using the Gao data, Applicants calculate the percentage of IL-4 produced by clone 81 in Lee as follows:

[1] Figure 2 of Gao indicates that a tobacco cell culture contained 600 grams wet cell weight per liter of medium on day 6 (data point indicated by an open circle on the topmost graph of Figure 2), and Figure 4 of Gao indicates that the same culture contained about 3.6 grams "total protein" per liter of medium on the same day (data point indicated by an open circle on the topmost graph of Figure 4). 3.6 grams protein per liter divided by 600 grams wet cell weight per liter equals 0.00599 grams protein per 1 gram cell weight. *See* Gao and Lee, Biotechnol. Progress 8(4):285-90 (1992) at page 287.

$$\frac{1 \text{ g calli cells}}{0.00599 \text{ g soluble protein}} \times \frac{1000 \text{ ng IL-4}}{1 \text{ g calli}} \times \frac{1 \times 10^{-9} \text{ g}}{1 \text{ ng}} = \frac{0.000001 \text{ g IL-4}}{0.00599 \text{ g soluble protein}}$$

$$\frac{0.000001 \text{ g IL-4}}{0.00599 \text{ g soluble protein}} \times 100 = 0.0167 \text{ \% of soluble protein is IL-4}$$

Accordingly, Applicants submit that Figure 11 of Lee does **not** teach an expression level even remotely close to 1% of total protein, soluble or insoluble. Applicants' calculations indicate that clone 81 from the Lee reference fails to teach protein expression at the level required by the claimed invention, and that Lee therefore fails to teach all of the elements of claims 1-7, 9, 10, 13, and 20.

The Office also asserts that Lee "expressly teaches that expression of over 1% of total protein is achievable", citing to Lee at column 1, lines 44-46. Office Action mailed August 6, 2002 at page 4 and Final Action at page 3. Applicants respectfully disagree. Merely stating a percentage of protein does not enable a skilled person to actually express protein at that level, and therefore Lee does not "expressly teach" the claimed invention. *See Symbol Technologies, Inc. v. Opticon, Inc.*, 935 F.2d 1569, 19 U.S.P.Q.2d 1241 (Fed. Cir. 1991). Whatever else Lee may teach or suggest, it does not teach or suggest a cytokine accumulating to a level greater than 1% of the total soluble protein. Thus, the Lee reference fails to disclose each and every limitation of the claimed invention.

With respect to the dependent claims, the Office goes on to assert that Lee anticipates claim 2 because Lee "purifies the cytokine using SDS-PAGE and TCA precipitation". Final Action at page 3. Applicants respectfully note that claim 2 includes the limitations of claim 1, and regardless of what Lee does or does not teach about

protein purification, Lee cannot anticipate claim 1 because it fails to teach every element of the invention of claim 2.

The Office then asserts that Lee teaches "a chimeric nucleic acid molecule which comprises the cloned plant cytokine gene under the control of a plant promoter sequence as well as signal sequences including signal sequences to the endoplasmic reticulum", and therefore anticipates claims 6-7, 9-10 and 13. Final Action at page 3. However, what Lee calls a "signal peptide" is not a "signal sequence" as defined by Applicants in their specification. Lee states that "[a] signal peptide from a secreted protein...can be used to target foreign polypeptides for secretion" and that the signal peptide acts "to direct the transfer of the growing polypeptide chain...for eventual secretion from the cell." *See* Lee at column 5, line 51 through column 6, line 5. In addition, the signal peptide of Lee is generally located at the N-terminus of a precursor polypeptide and is commonly cleaved from the precursor polypeptide to produce a "mature" polypeptide lacking the signal peptide. *Id.* In contrast, the instant specification includes signal sequences that "will direct the cytokine of interest to a sub-cellular location (*e.g.*, cytosol, endoplasmic reticulum, plastid, and chloroplast)". *See* specification at page 27, line 29 through page 28, line 1. Therefore, Lee does not teach the signal sequence of the instant specification and, as such, does not anticipate these claims.

Whatever else Lee may teach or suggest, it does not teach or suggest a cytokine accumulating to a level greater than 1% of the total soluble protein. Applicants respectfully submit that Lee does not teach all elements of the pending claims and, therefore, that Lee does not anticipate claims 1-7, 9-10, 13 and 20.

Claims 3-5 and 91-93

The Examiner alleges that "Lee teaches a method for producing a cytokine, such as IL-4, which is free from amino acid modifications or novel glycosylation". Final Action at page 2. Therefore, the Examiner argues that Lee anticipates claims 3-5 and 91-93, which recite limitations that the expressed cytokine is free from amino acid

modification (claims 3-4, 91), or that the cytokine is free of novel glycosylation (claims 5, 92-93). Applicants respectfully traverse this rejection.

As previously stated in Applicants' First Response (dated December 6, 2002), despite whatever else it may teach or suggest, Lee fails to teach or suggest production of a cytokine that is free from amino acid modifications or novel glycosylation. However, the Office contends that Figure 13 of the Lee reference, which depicts a Western blot, proves that "IL-4 has [the] same molecular weight as recombinant human IL-4." Final Action at page 2. The Office argues that "if the protein mobility is the same between unmodified and unglycosylated control protein and the plant expressed protein, that is strong evidence that the protein in the plant is also unmodified and unglycosylated." *Id.* at pages 7-8. Apparently solely on the basis of the Western blot, the Office concludes that Lee anticipates the claimed invention. Applicants disagree.

Applicants respectfully submit that the Office is incorrect in the assumptions it has made based on a Western blot's showing of similar protein mobility between a control protein and a plant expressed protein. The Office argues that similar molecular weight, as shown by protein mobility on a Western blot, is "strong evidence that the protein in the plant" has the same structure as the control protein, *e.g.*, "is also unmodified and unglycosylated." Final Action at pages 7-8. This is not true. Applicants submit that a 10% SDS-PAGE Western blot is simply unable to resolve the exact amino acid composition or glycosylation of a protein. Furthermore, the argument advanced by the Office is not "evidence" but rather speculation on the part of the Office, and is contrary to the teachings in the art and the present specification.

The art teaches that molecular weight does not necessarily correlate with glycosylation patterns, *e.g.*, a glycosylated recombinant protein can have the same molecular weight as its deglycosylated wild-type protein. *See, e.g.*, Kusnadi *et al.*, Biotechnology and Bioengineering, vol. 56, No. 5, pages 473-84 (1997) (previously submitted in Applicants' IDS of August 29, 2001). In addition, Example 9 of the present specification illustrates how two proteins appearing to have the same mobility on a Western blot (Fig. 28), and in fact having very similar molecular weights, were revealed

to have different glycosylation patterns when mass spectroscopy was used to examine their structure. Specification at pages 54-56, and particularly page 55 line 18 through page 56, line 4. Furthermore, upon sequencing of a plant expressed protein in Example 9, Applicants showed that it possessed an amino acid modification (proline to hydroxyproline) despite having virtually the same molecular weight as the control protein. *Id.* at page 55, lines 10-17.

Furthermore, the standard used by Lee to evaluate protein mobility is unclear. The text of Lee reports that human IL-4 has a size of about 19.5 kDa (Lee at col. 20, line 24), but the human IL-4 standard actually used in Figure 13 (lane 1) is a methionyl form of *E. coli*-produced rhu-IL-4 with a reported size of 14 kDa. *See* Lee at col. 20, lines 61-64; R&D Systems Catalog Number 204-IL (attached hereto as Exhibit C). In Figure 13 of Lee, protein from the media (lane 3) shows two bands which "bracket" the standard of lane 1, and internal cellular content (lane 5) shows multiple bands including a band that appears to have similar mobility to the standard of lane 1. It is not clear from Lee what the expected mobility for an unmodified protein should be, and Applicants therefore submit that even if it were theoretically possible to determine protein structure and glycosylation patterns from a Western blot, Lee has not provided sufficient information against which to compare its experimental data.

Accordingly, Applicants respectfully submit that the Office is incorrect in the assumptions it has made based on a Western blot's showing of similar protein mobility between a control protein and a plant-expressed protein. Applicants raised this issue in their First Response, where they asserted that a Western blot does not reveal amino acid composition or protein structure, and therefore cannot provide accurate information regarding potential amino acid modifications or glycosylation changes. Applicants' First Response, at page 7. In the Final Action, however, the Office merely reiterated its prior assumptions, and presented no evidence, either in the form of a reference or an affidavit under 37 C.F.R. § 104(d)(2), to support the assumptions which Applicants questioned. Applicants must assume, from the lack of evidence provided in support of the Examiner's interpretation of the Western blot, that the Examiner is relying on his own personal

knowledge to interpret Lee, and therefore they respectfully request that the Examiner provide an affidavit supporting his interpretation pursuant to 37 C.F.R. § 104(d)(2).

On the basis of the foregoing, Applicants submit that the Office has failed to meet its *prima facie* burden of proof, because it has not shown that Lee teaches all of the elements of claims 1-7, 9, 10, 13, 20, and 91-93. Accordingly, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. §102.

2. Rejections under 35 USC § 103

Lee in view of Boone et al.

Claim 22 is rejected under 35 USC § 103 (a) as being allegedly unpatentable over Lee in view of Boone *et al.* (U.S. Patent 5,849,883) (hereafter "Boone"). Office Action mailed August 6, 2002 at page 5 and Final Action at page 4. Applicants respectfully traverse this rejection.

Applicants note that, for at least the reasons discussed above, Lee is deficient as a primary reference because it fails to teach or suggest the limitations of claim 1 upon which claim 22 depends. For example, Lee fails to teach or suggest the accumulation of an expressed cytokine to a level greater than 1% of the total soluble protein. However, Applicants' argument below will demonstrate that Boone does not supplement the deficiencies of Lee, and that these references taken alone or together do not teach or suggest the invention of claim 22. Therefore, Applicants respectfully assert that the Examiner has not provided a *prima facie* case of obviousness under 35 U.S.C. § 103 and respectfully request withdrawal of the rejection.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed

combination and the reasonable expectation of success must both be found in the prior art, and not based on the applicant's disclosure. *In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Applicants respectfully assert that the Office has failed to establish a *prima facie* case of obviousness because the Office has not provided an adequate explanation of the suggestion or motivation to combine the teachings of Lee and Boone. The Office alleges that it would have been obvious to one of ordinary skill in the art at the time the invention was made to express G-CSF in plants using the method of Lee because Boone purportedly suggests plant cell expression of G-CSF. Final Action at page 5. Applicants respectfully disagree.

"[Lee] provides compositions and methods for increasing the expression and recovery of heterologous polypeptides secreted by cultured plant cells." *See* Lee at column 3, lines 65-67. Boone ostensibly describes lysing a microorganism and separating insoluble material containing G-CSF from the soluble proteinaceous material. *See* Boone at column 28, line 60-65. The Office's alleged suggestion to combine these references is a statement in Boone at column 10, lines 6-9 that the "polypeptide are also characterized by being the product of chemical synthetic procedures or of prokaryotic or eukaryotic host expression (e.g., by bacterial, yeast, higher plant, insect and mammalian cells in culture)...". Applicants submit that Boone at the most suggests that it might be "obvious-to-try" to express G-CSF in a plant, but that Boone does not contain any suggestion or motivation to use the method of Lee to achieve the expression levels of claim 22. Furthermore, Boone does not teach or suggest how to accomplish the claimed expression in plants, that expression in plants would work at all, or that there would be any reasonable expectation of success in accumulating G-CSF at a level greater than 1 % of the total soluble protein in plants using the method of Lee.

Neither Lee nor Boone, whether taken together or separately, suggest or motivate using the method of Lee with the G-CSF genes of Boone to achieve the claimed invention. There is no suggestion in either reference regarding what the expression level of G-CSF in plant cells would be. Therefore, Applicants argue that there is no suggestion

or motivation, either in the references themselves or in the knowledge generally available to one of skill in the art, to modify or combine the teachings of Lee with Boone to accumulate an expressed cytokine to a level greater than 1% of the total soluble protein in a plant host system.

Applicants also respectfully assert that the Office has failed to establish a *prima facie* case of obviousness because there would have been no reasonable expectation of success in combining the teachings of Lee and Boone. The Office has argued that Boone suggests "expression of G-CSF in plants." Final Action at page 5. However, one of skill in the art would have no reasonable expectation that the G-CSF gene would function sufficiently well in plants to accumulate to a level greater than 1% of the total soluble protein based on the methods and compositions for increasing the expression and recovery of secreted polypeptides disclosed by Lee. Certainly, Lee's failure to achieve the claimed expression levels in plants would not create an expectation that Lee's method would be successful with other genes.

Finally, Applicants respectfully assert that the Office has failed to establish a *prima facie* case of obviousness because, even when combined, the teachings of Lee and Boone do not teach or suggest all the instant claim limitations. The law requires that an anticipatory reference must teach or suggest all of the claim limitations. *In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). As stated above, neither Lee nor Boone teach or suggest the expression of a cytokine to a level greater than 1% of the total soluble protein in a plant host system.

For the foregoing reasons, Applicants respectfully assert that the Office has failed to establish a *prima facie* case of obviousness over Lee in view of Boone. Therefore, Applicants respectfully request withdrawal of the rejection of claim 22 under U.S.C. § 103 for purportedly being unpatentable over Lee in view of Boone.

Lee in view of Schouten et al.

Claim 8 is rejected under 35 USC § 103 (a) as being allegedly unpatentable over Lee in view of Schouten *et al.* (FEBS Lett. (1997) 415:235-241) (hereafter "Schouten").

Office Action mailed August 6, 2002 at page 7 and Final Action at page 6. Applicants respectfully traverse this rejection.

Again, Applicants note that, for at least the reasons discussed above, Lee is deficient as a primary reference because it fails to teach or suggest each and every limitation of claim 1 upon which claim 8 depends. For example, Lee fails to teach or suggest the accumulation of an expressed cytokine to a level greater than 1% of the total soluble protein. However, Applicants' argument below will demonstrate that Schouten does not supplement the deficiencies of Lee and that these references taken alone or together do not teach or suggest the invention of claim 8. Therefore, Applicants respectfully assert that the Examiner has not provided a *prima facie* case of obviousness under 35 U.S.C. § 103 and respectfully request withdrawal of the rejection.

The Office has not provided an adequate explanation of the suggestion or motivation to combine the teachings of Lee and Schouten. Lee teaches the addition of a signal peptide (or leader sequence) of a secreted protein onto a heterologous polypeptide to target that foreign polypeptide for secretion from plant cells. *See* Lee at column 5, line 65 through column 6, line 5. Schouten is directed to the expression of single-chain antibody fragments in plant cytosol. Schouten at page 235. Therefore, Applicants argue that there is no suggestion or motivation, either in the references themselves or in the knowledge generally available to one of skill in the art, to modify or combine the teachings of Lee with Schouten in order to accumulate an expressed cytokine with a KDEL sequence to a level greater than 1% of the total soluble protein in a plant host system.

Applicants respectfully assert that the Office has failed to establish a *prima facie* case of obviousness because there would have been no reasonable expectation of success, at the time the invention was made, in combining the teachings of Lee and Schouten. Lee purportedly teaches the addition of a signal peptide to the ***N-terminus*** of a precursor protein to direct the heterologous protein for further processing. *See* Lee at column 5, line 65 through column 6, line 5. The signal peptide is commonly cleaved from the precursor polypeptide to produce a "mature" polypeptide lacking the signal peptide and

the mature polypeptide is eventually secreted from the cell. *Id.* Schouten, however, teaches the use of a KDEL sequence fused to the ***C-terminus*** for ER expression.

The Examiner asserts that an ordinary practitioner would have been motivated to use the KDEL retention signal of Schouten in the expression method of Lee. Final Action at page 6. However, the KDEL sequence of Schouten and the signal peptide of Lee have different functions and are attached to different portions of a polypeptide. Neither Lee nor Schouten disclose the effect of a combination of a N-terminal signal peptide targeting secretion and a C-terminal KDEL ER retention signal on a heterologous polypeptide, *e.g.*, where the polypeptide would reside or what the expression levels of such a polypeptide would be. Therefore, one of skill in the art would have no reasonable expectation that use of the KDEL retention signal of Schouten in the expression method of Lee would successfully accumulate a cytokine to a level greater than 1% of the total soluble protein in a plant host system.

Applicants also respectfully assert that the Office has failed to establish a *prima facie* case of obviousness because neither Lee nor Schouten, either combined or taken together, discuss or suggest all of the claim limitations. The law requires that an anticipatory reference must teach or suggest all of the claim limitations. *In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). As described above, whatever else Lee or Schouten disclose, neither reference teaches expression of a cytokine to a level greater than 1% of the total soluble protein in a plant host system. Furthermore, neither reference teaches expression of a G-CSF to a level greater than 1% of the total soluble protein in a plant host system. Hence, the cited references taken alone or in combination do not teach, suggest, or make obvious the present invention. In light of these remarks, Applicant respectfully requests withdrawal of this rejection of claim 8 under 35 U.S.C. §103, for purportedly being unpatentable over Lee in view of Schouten.

Accordingly, on the basis of the foregoing, Applicants respectfully request that the rejection of claims 8 and 22 under 35 U.S.C. §103 be withdrawn.

CONCLUSION

It is believed that the present claims are in immediate condition for allowance. Accordingly, Applicants respectfully request that the Examiner pass the application to issue. In the event that any extensions of time are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned. Applicants do not believe any fees are due in conjunction with this filing. However, if any fees under 37 C.F.R. §§ 1.16 or 1.17 are required in the present application, including any fees for extensions of time, then the Commissioner is hereby authorized to charge such fees to Arnold & Porter Deposit Account No. 50-2387, referencing matter number 18337.006.

Respectfully submitted,

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